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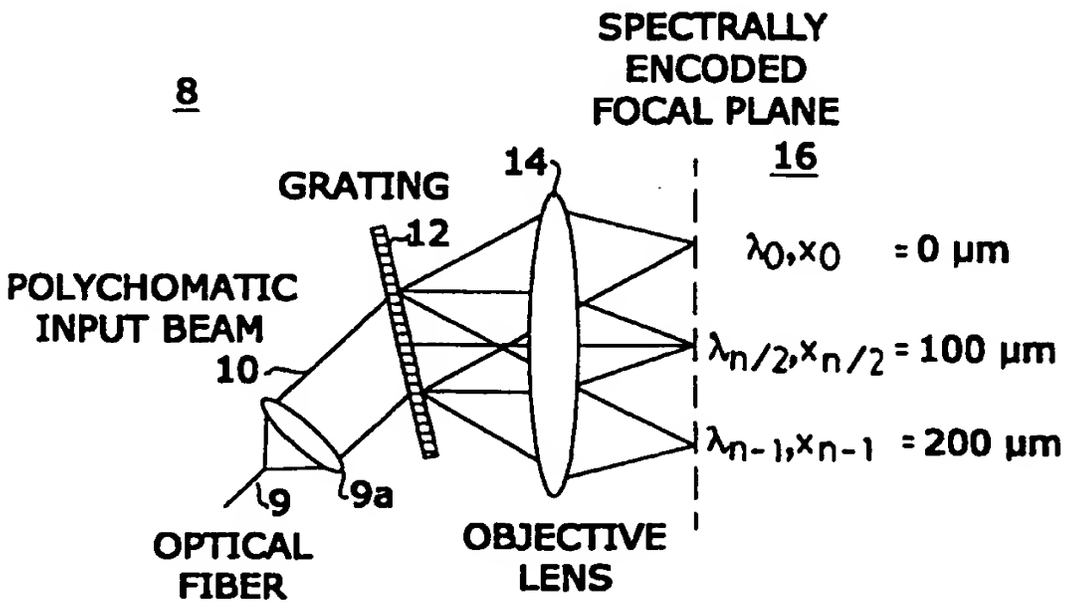
(54) Title: CONFOCAL MICROSCOPY WITH MULTI-SPECTRAL ENCODING



(57) Abstract

A scanning confocal microscopy system, especially useful for endoscopy with a flexible probe which is connected to the end of an optical fiber (9). The probe has a grating (12) and a lens (14) which delivers a beam of multi–spectral light having spectral components which extend in one dimension across a region of an object and which is moved to scan in another dimension. The reflected confocal spectrum is measured to provide an image of the region.

CONFOCAL MICROSCOPY WITH MULTI-SPECTRAL ENCODING

This application claims the priority benefit of U.S. Provisional Application No. 60/076,041, filed 26 February 1998.

Description

The present invention relates to systems (method and apparatus) for confocal microscopy for the examination or imaging of sections of a specimen of biological tissue, and particularly to such systems using multi-spectral illumination and processing of multi-spectral light.

Currently, the use of fast scanning confocal microscopy is limited to accessible surfaces of the skin and the eye. The reason for this is that the only reliable methods for optical scanning must be performed in free space. In addition, the size of these optical scanners prohibit their use in small probes such as endoscopes or catheters. It is a feature of the invention to miniaturize the fast scanning mechanism and increase the number of medical applications of confocal microscopy to include all surfaces of the body, gynecologic applications, probe-based applications, and internal organ systems.

Multi-spectral light was proposed for use in confocal microscopy, but only for imaging vertically-spaced regions of a body under examination. See B. Picard, U.S. Patent No. 4,965,441, issued October 25, 1990. An interferometer using a grating to obtain multi-spectral light which is resolved in the interferometer to obtain a spectroscopic image is disclosed in A. Knuttal, U.S. Patent 5,565,986, issued October 15, 1996. A lens having a color separation grating which obtains a multi-spectral light is disclosed in U.S. Patent No. 5,600,486, issued February 4, 1997. Such multi-spectral proposals are not effective for high resolution imaging using a compact, flexible probe. A confocal microscope system according to this invention can be miniaturized and incorporated into a compact probe. In addition, by allowing light delivery through a single optical fiber, the probe may also be easily incorporated into catheters or endoscopes. Thus, a confocal microscope in accordance with

Fig. 6 is a schematic diagram showing cross-sectional image formation by scanning the optical fiber or the objective lens along the z axis using a system embodying the invention.

Fig. 7 is another schematic diagram of a system embodying the invention wherein optical zoom is achieved by moving the focus of an intermediate lens in and out of the image plan of the objective.

Referring now to the figures, multi-spectral encoding for confocal microscopy uses a broad bandwidth source 10 as the input to the microscope. In the probe 8 of the microscope, the source spectrum provided via an optical fiber 9 is dispersed by a grating 12 and focused by an objective lens 14 onto the sample 16. A lens 9a is preferably disposed between the optical fiber 9 and the grating 12 to collimate the light from the optical fiber, as shown in Fig. 1, however, lens 9a may be removed. The spot for each wavelength is focused at a separate position, x, on the sample (Fig.1). The reflectance as a function of transverse location is determined by measuring the reflected confocal spectrum from the sample 16 returned from probe 8.

The number of wavelengths or points that may be resolved is determined by:

$$\frac{\lambda}{\delta\lambda} = mN, \tag{1}$$

where λ is the center wavelength, $\delta\lambda$ is the bandwidth of the spectrum, N is the number of lines in the grating 12 illuminated by the polychromatic input beam 10, and m is the diffraction order. If the total bandwidth of the source is $\Delta\lambda$, the number of resolvable points, n is defined by:

$$n = \frac{\Delta\lambda}{\delta\lambda} \tag{2}$$

reference arm 22 and measuring the cross-correlation output 30 from the interference spectrometer due to the interference of the reflected light from the sample and reference arms 18 and 22, respectively (Fig. 4). The advantages to this type of spectroscopic detection include the ability to achieve higher spectral resolutions than direct detection methods, efficient use of the returned light, inherent modulation of the reference arm 22 by the Doppler shift of the moving mirror 28, and the capability to extract both reflectance and phase data from the sample 16. The ability to extract phase data from the sample may allow detection of refractive index as a function of transverse position, x, which is useful to reveal the molecular composition of the sample as well as provide an additional source of image contrast other than the reflectivity of the sample specimen 16. Finally, interferometric detection has the potential to allow elimination of high order multiple scattering from the confocal signal by coherence gating.

Consider finally image formation. The multi-spectral encoding of the transverse location, x, allows the performance of a one-dimensional raster scan. To obtain an image, a scan of another axis must be performed, which is usually slower. Methods of accomplishing this slow scanning of the y axis include moving the optical fiber 9 in the y direction (Fig. 5B), or rotating the entire probe 8 around the optical fiber axis either in a forward scanning configuration (Fig. 5C) or a side-firing configuration (Fig. 5D). Cross-sectional images may be created by scanning the optical fiber 9 or the objective lens 14 along the z axis (Fig. 6). Finally, a zoom mode may be created by scanning the optical fiber 9 (or another lens 32 between grating 12 and objective lens 14), in and out of the image plane of the objective lens (Fig. 7). Both linear motion along the y or z axis and rotation are easily accomplished in a compact probe by use of piezoelectric transducers. As shown in FIG. 5A, signals may be received by a computer 34 from spectroscopic detector 32 by a spectrometer (such as described in connection with FIG. 3) or Fourier transform (such as described connection with

Claims

1. A confocal microscope system which comprises a probe movable into a body region of interest, said probe having means for illuminating said region with a confocal spectrum of light extending along one dimension, means for obtaining an image of the region of the specimen by moving said spectrum along another dimension and measuring the reflected confocal spectrum of said light.

2. The system according to Claim 1 wherein said probe is mounted on the end of a flexible, light-conducting member.

3. The system according to Claim 2 wherein said member is an optical fiber.

4. The system according to Claim 3 wherein said fiber is rotatable or translatable to move said probe in said another dimension.

5. The system according to Claim 1 wherein said means for moving said spectrum comprises means for moving an image plane containing said spectrum optically or by physically moving said probe.

6. The system according to Claim 5 wherein said probe is moved physically to scan said spectrum in said another dimension and said probe has means for optically moving said image plane to scan in still another direction, thereby enabling 3-D imaging.

7. The system according to Claim 1 wherein said means for obtaining said image comprises heterodyne detection means.

8. The system according to Claim 7 wherein said heterodyne detection means includes an interferometer.

9. The system according to Claim 8 wherein said interferometer has a sample arm terminated by said probe, a reference arm terminated by a mirror, an output arm having a spectroscopic detector, an input arm having a source of polychromatic illumination, and a beam splitter for directing light from said source to said sample and reference arms and directing interfering light containing said reflected confocal spectrum into said output arm.

20. The system according to Claim 17 further comprising an optical fiber which provides said light from said source to said producing means, and provides said returned light from said focusing and receiving means to said detecting means.

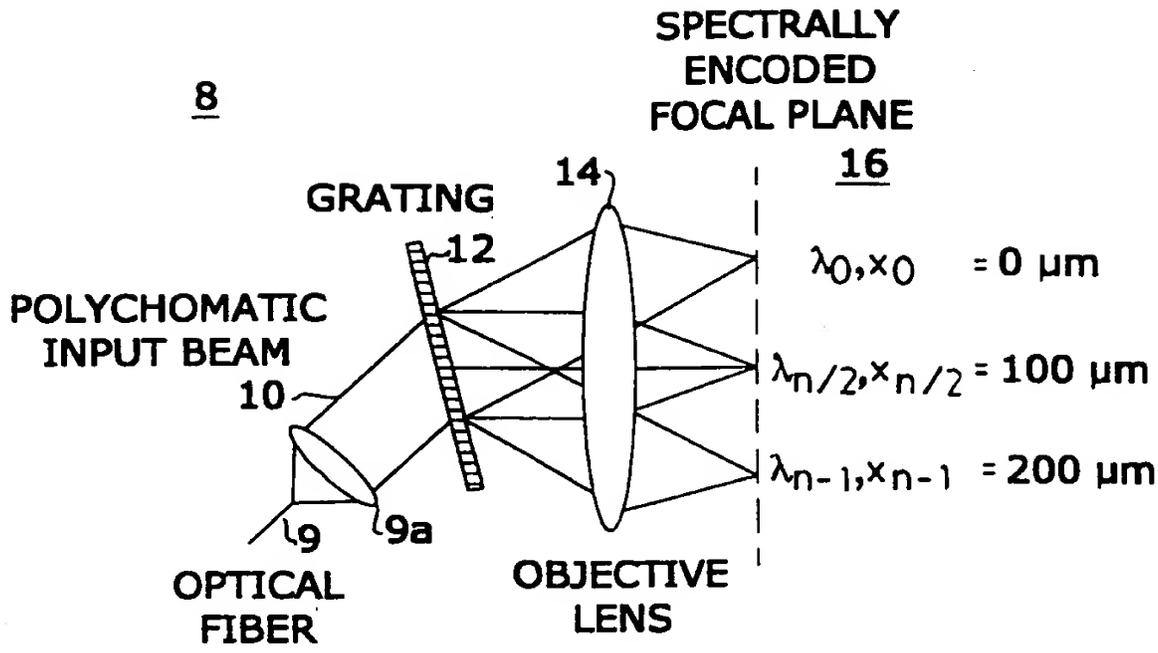

8
SPECTRALLY ENCODED FOCAL PLANE
16
14
GRATING
12
POLYCHOMATIC INPUT BEAM
10
λ0,x0 = 0 μm
λn/2,xn/2 = 100 μm
λn-1,xn-1 = 200 μm
9
9a
OPTICAL FIBER
OBJECTIVE LENS

FIG.1

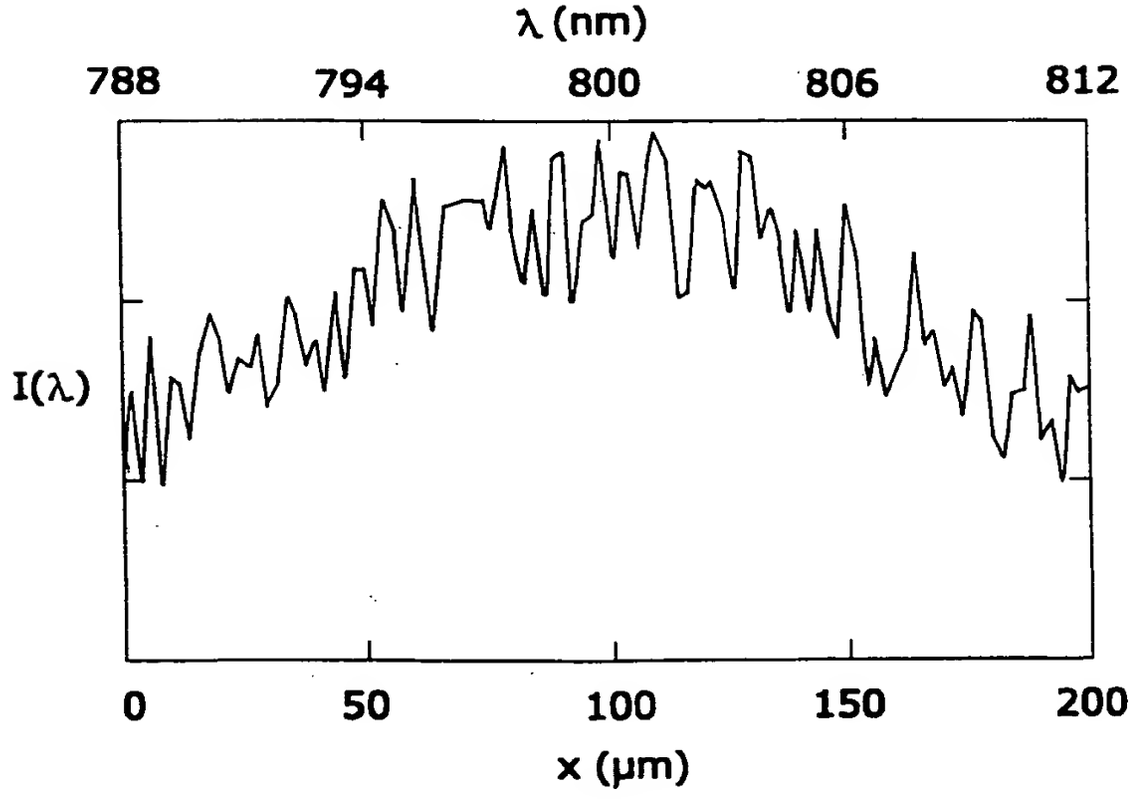

λ (nm)
788
794
800
806
812
I(λ)
0
50
100
150
200
x (μm)

FIG.2

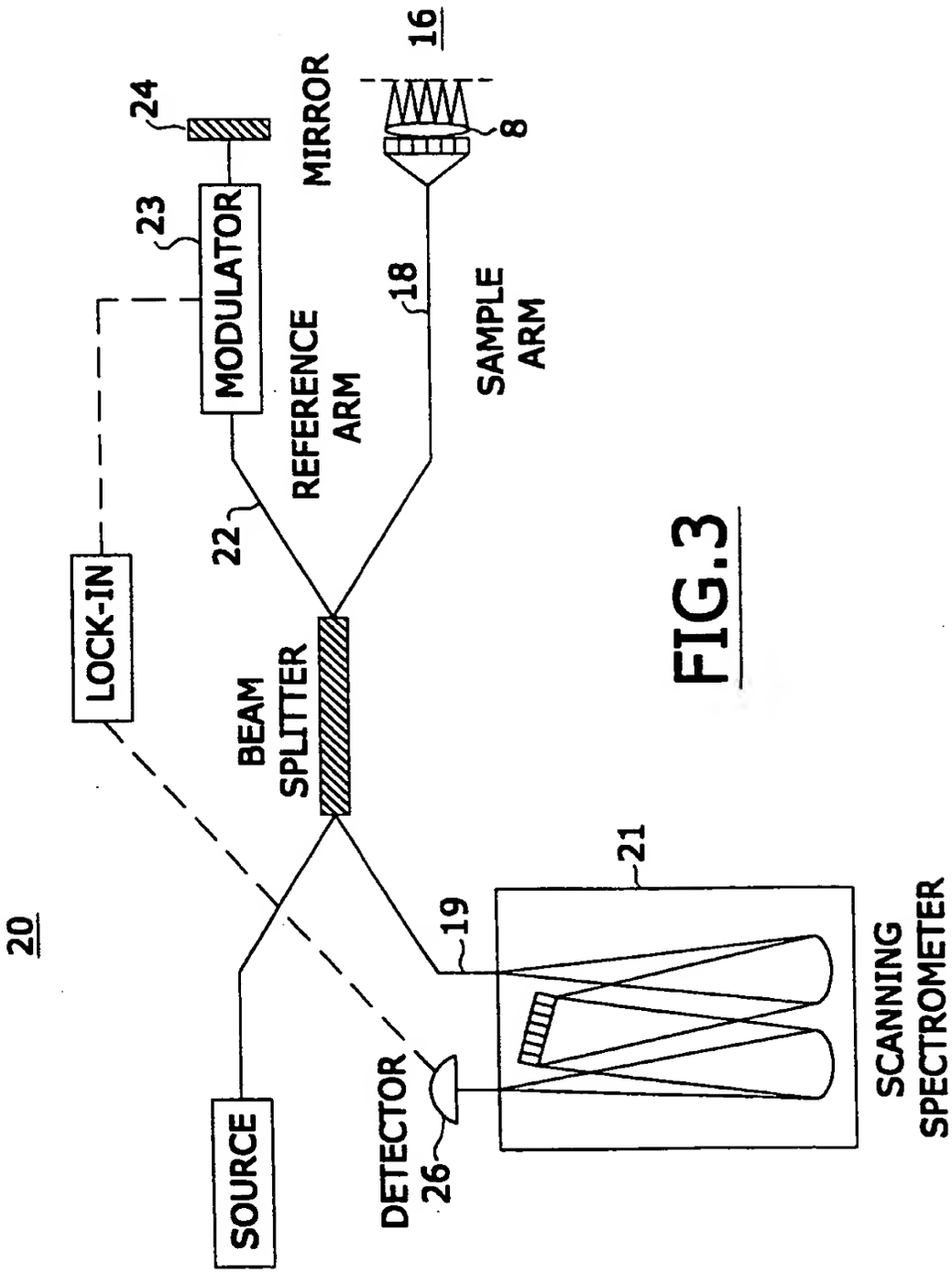

20
LOCK-IN
SOURCE
BEAM SPLITTER
MODULATOR
REFERENCE ARM
SAMPLE ARM
MIRROR
DETECTOR
SCANNING SPECTROMETER
16
24
23
22
18
8
19
21
26

FIG.3

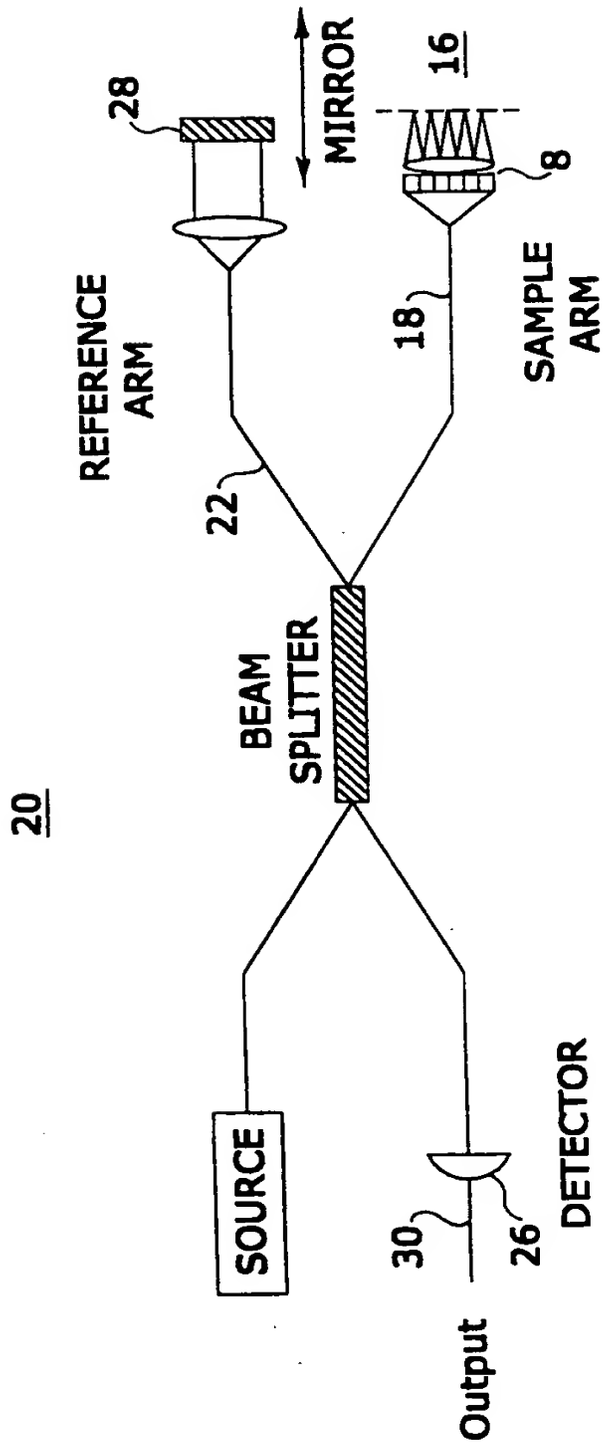

20
BEAM SPLITTER
REFERENCE ARM
SAMPLE ARM
MIRROR
SOURCE
DETECTOR
Output
22
18
16
28
8
30
26

FIG.4

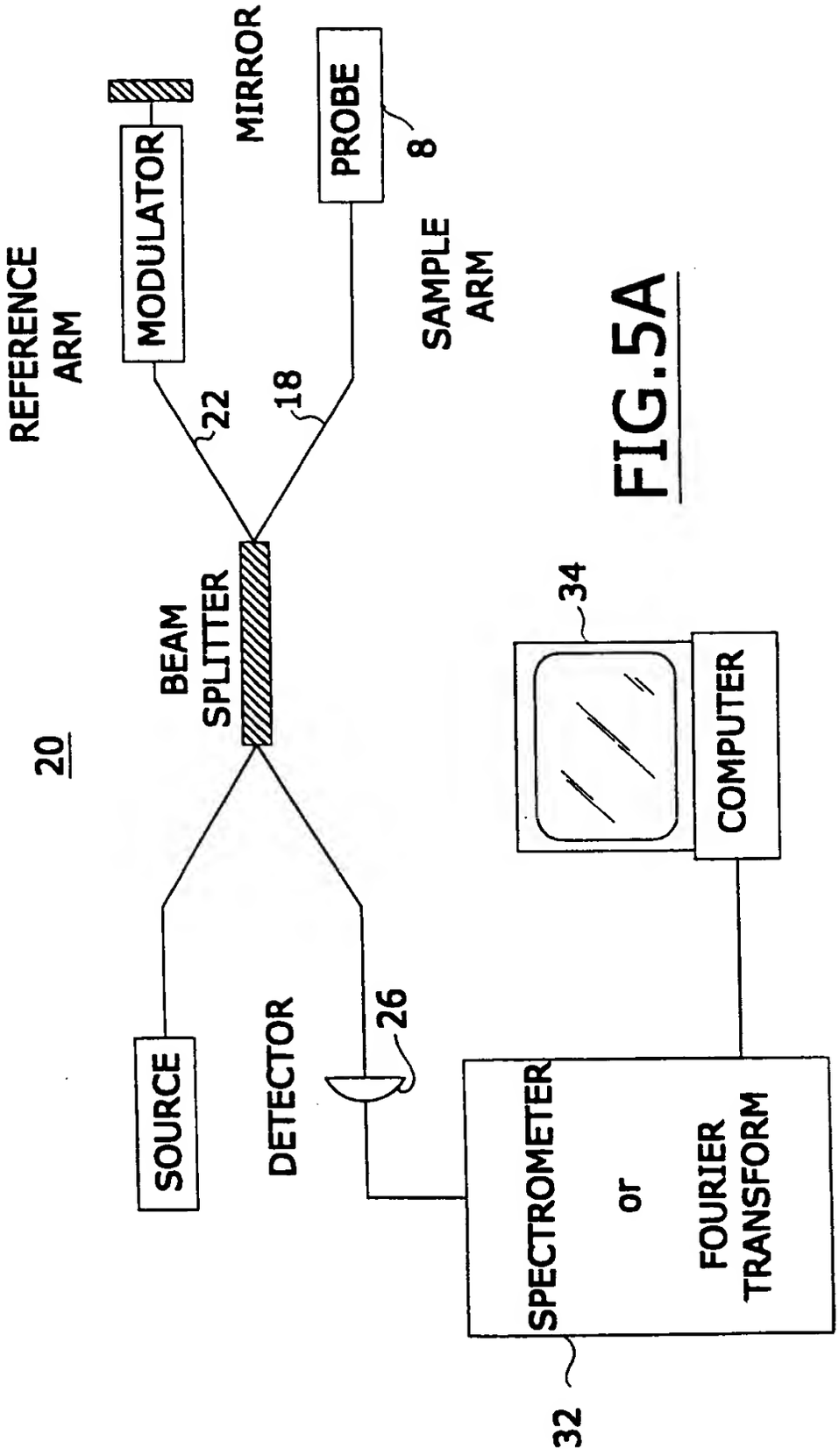

REFERENCE ARM
MODULATOR
MIRROR
PROBE
8
SAMPLE ARM
22
18
20
BEAM SPLITTER
34
COMPUTER
SOURCE
DETECTOR
26
SPECTROMETER or FOURIER TRANSFORM
32

FIG.5A

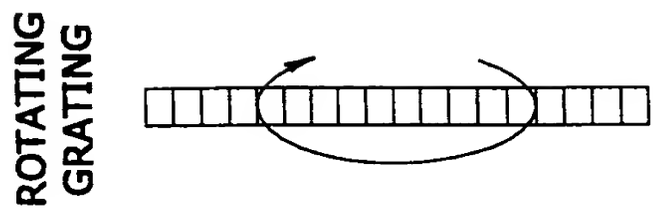
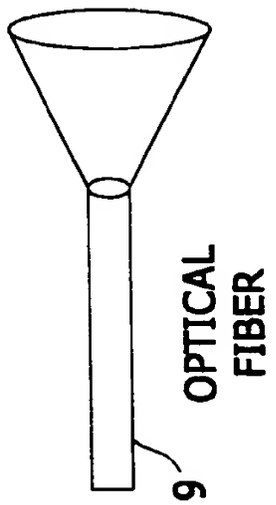
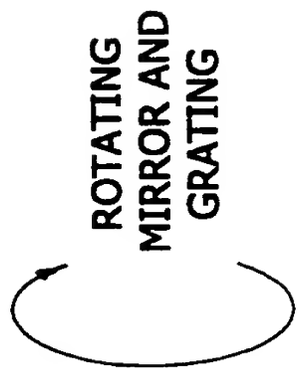


FIG.5C





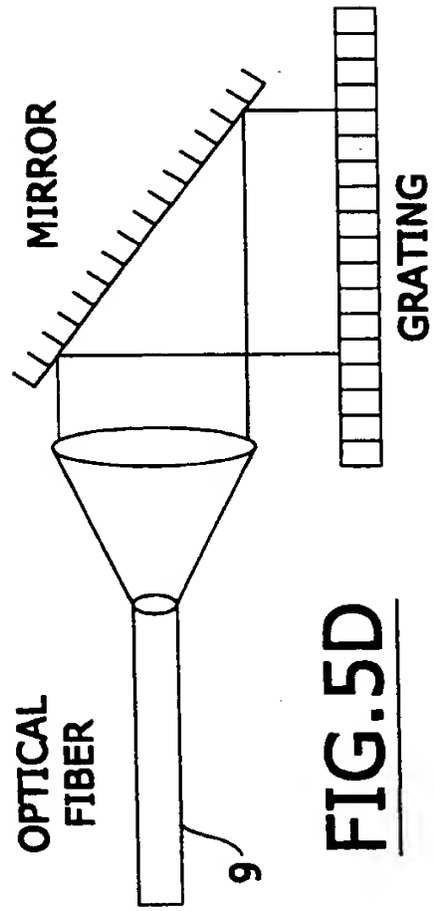


FIG.5D

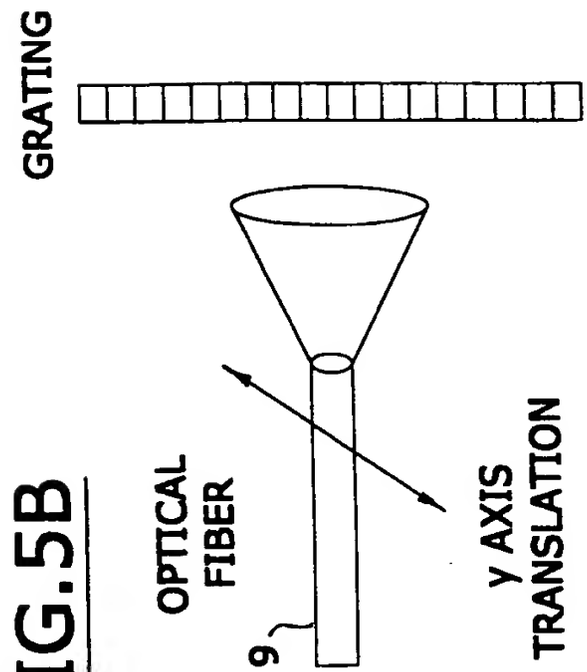


FIG.5B

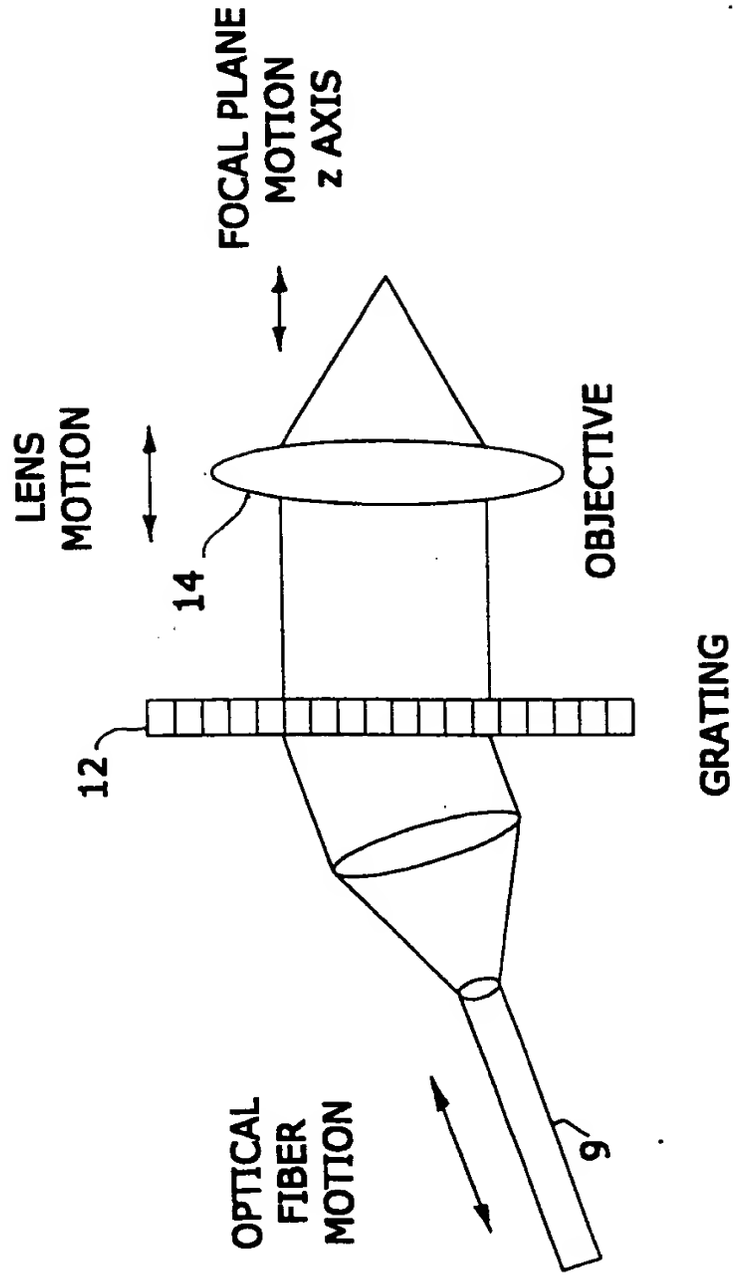

FOCAL PLANE MOTION z AXIS
LENS MOTION
14
OBJECTIVE
12
GRATING
OPTICAL FIBER MOTION
9

FIG.6

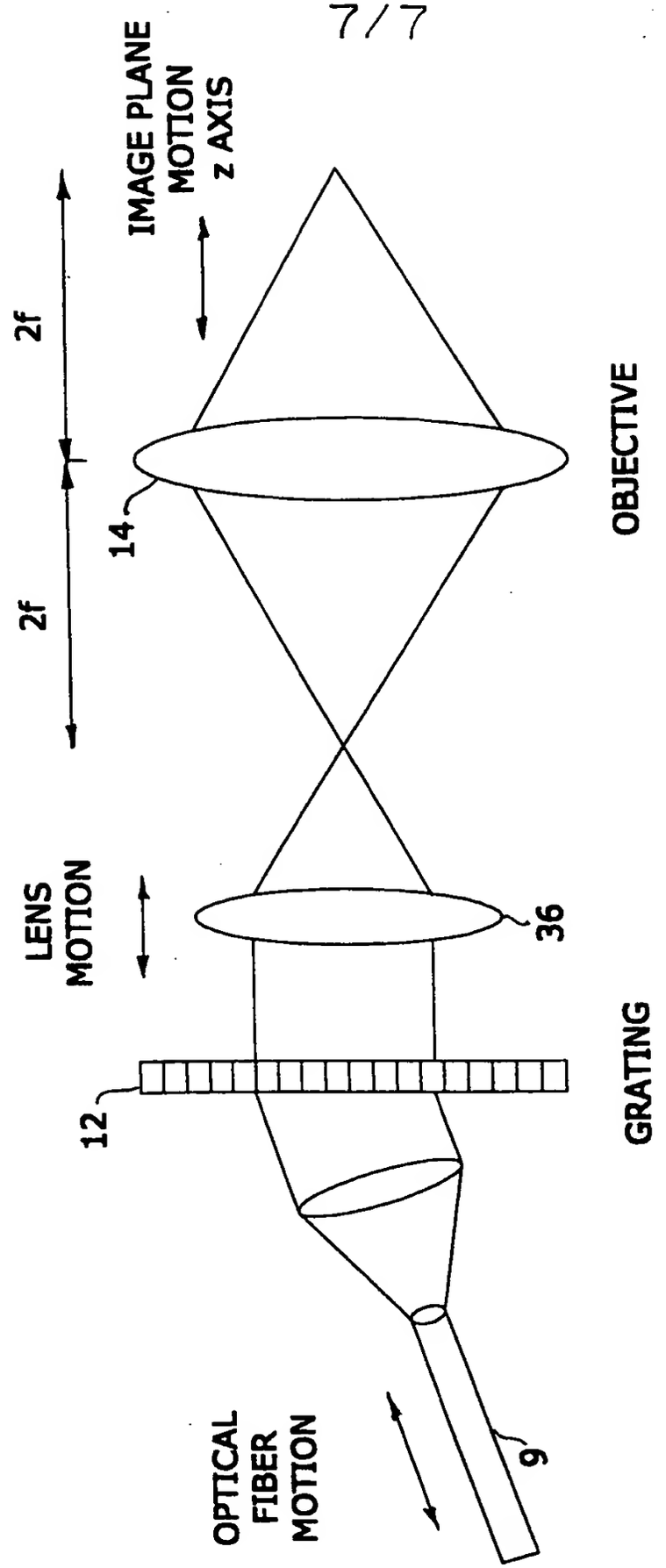


IMAGE PLANE MOTION z AXIS
2f
2f
14
OBJECTIVE
LENS MOTION
36
12
GRATING
OPTICAL FIBER MOTION
9

FIG.7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04356

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G02B 21/00
US CL :359/368
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 359/368,389

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: confocal, probe, microscope or endoscope, grating, diffraction

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,450,203 A (PENKETHMAN) 12 September 1995 (12/09/95), see entire document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 JUNE 1999	Date of mailing of the international search report 20 JUL 1999
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